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Marine Environmental Research 54 (2002) 793–797

MARINE ENVIRONMENTAL RESEARCH

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Seasonal variation in lysosomal destabilization in oysters, *Crassostrea virginica*

Amy H. Ringwood[a,*], Jennifer Hoguet[b], Charles J. Keppler[a]

[a] *Marine Resources Research Institute, SC Department of Natural Resources, 217 Fort Johnson Road, Charleston, SC 29412, USA*

[b] *University of Charleston, SC, USA*

Abstract

Lysosomal destabilization assays have been used as valuable biomarkers of pollutant exposures in a variety of bivalve and fish species. The responses of oysters, *Crassostrea virginica*, deployed at and native to various reference and degraded sites were evaluated for lysosomal destabilization during both summer and winter seasons. In both native and deployed oysters, lysosomal destabilization rates tended to be higher during the winter at both reference and polluted sites. There are at least two hypothetical explanations. Greater lysosomal destabilization rates may be related to physiological changes associated with mobilization of nutrient reserves during the winter and gametogenesis. However, lysosomal destabilization in deployed oysters was correlated with tissue metal concentrations. These data also support a second hypothesis that seasonal differences in physico-chemical factors (such as reduced levels of acid volatile sulfides) may increase the bioavailability of metals during the winter so that adverse effects are more pronounced.

Keywords: Lysosomes; Oysters; Metals

Lysosomal destabilization assays have been used as valuable biomarkers of pollutant exposures in a variety of bivalve species (Lowe & Fossato, 2000; Moore, 1982; Regoli, Nigro, & Orlando, 1998; Ringwood, Conners, & Hoguet, 1998). Important issues associated with validation and interpretation of biomarker responses are identification of seasonal differences and appropriate characterization of "normal" ranges (Ringwood et al., 1999). If seasonal differences are observed, the question is, are they related to physiological differences or seasonal differences in bioavailability and bioaccumulation of contaminants? While metabolic rates may tend to be lower

* Corresponding author. Tel.: +1-843-762-5404; fax: +1-843-762-5110.
E-mail address: ringwooda@mrd.dnr.state.sc.us (A.H. Ringwood).

0141-1136/02/$ - see front matter
PII: S0141-1136(02)00181-2

during the cooler months, the bioavailability of contaminants could actually be greater since acid volatile sulfides (AVS) and organic carbon levels tend to be lower during the winter months (Besser, Ingersoll, & Giesy, 1996). Likewise, since metabolic rates during the warmer months are higher, it may be expected that bioaccumulation would be higher, but higher AVS levels and organic loads may reduce bioavailability.

The results of seasonal studies with oysters, *Crassostrea virginica*, are reported here in which lysosomal responses of native and deployed juvenile oysters were evaluated during summer (June–August, temperature range 25–30 °C) and winter (January–March, temperature range 10–15 °C) seasons. The deployed juvenile oysters (3–4 cm in height) were placed in cages for 28–30 days. Sites at which native oysters were collected were not always the same sites used for the caging studies. Data from 1998 and 2000 are presented for the deployed oyster studies, and data from 1999 and 2000 are presented for native oysters. Native and deployed oysters were collected from the field, held in site water overnight, and then lysosomal destabilization assays were conducted with hepatopancreas tissues. Lysosomal destabilization assays were conducted as previously described (Ringwood et al., 1998). The concentrations of metals in the tissues were also determined for the oysters deployed in situ in 1998. Three to four composite samples (each composed of 5–10 oysters) were lyophilized, ground to a powder, solubilized in 70% nitric acid using microwave digestion techniques, and the concentrations of Zn, Cu, and Cd were determined by ICP or AAS.

Although there were some cases where there were no significant differences between winter and summer rates of lysosomal destabilization, there was a general tendency for higher rates during the winter, particularly at the more heavily contaminated sites (Fig. 1). This was true for deployed as well as native oysters at a variety of sites from different years. Most of the sites for the native oyster studies were relatively uncontaminated sites, and the purpose of these studies was to establish normal base-line rates for reference conditions. For the summer period, these results were consistent with earlier conclusions that lysosomal destabilization rates $>30\%$ were indicative of contaminant exposure. However, when these kinds of analyses were conducted during cooler winter periods, many sites, even relatively uncontaminated sites were higher than 30%.

The important question is, should the ''normal'' lysosomal destabilization levels for winter oysters be defined as higher than that used for summer oysters? This depends on whether the increased levels observed during the winter are associated with normal physiological differences, or if the higher levels provide important, perhaps subtle, information about habitat condition. Significant relationships between tissue metal levels and lysosomal destabilization rates were observed (Fig. 2). It should also be noted from Fig. 2 that the highly significant regressions were not the result of distinct winter and summer clusters, but rather that lysosomal destabilization rates were significantly related to metal tissue levels, regardless of season. There is a general tendency for the AVS levels and total organic carbon concentrations to be lower during the winter time than during the summer (data not shown), so increased bioavailability would be predicted. On a site-specific basis,

there was a tendency for deployed oysters to accumulate higher tissue metal concentrations in the winter than in the summer, supporting the model of increased bioavailability during the winter.

While we cannot eliminate the possibility that seasonal changes in physiological conditions may contribute to seasonal differences in lysosomal destabilization rates, these results suggest that tissue metal concentrations may be higher during the winter due to increased bioavailability of metals or other pollutants during cooler periods. These results further suggest that contaminant effects may be more readily detected during the winter period. Highly contaminated sites showed significant

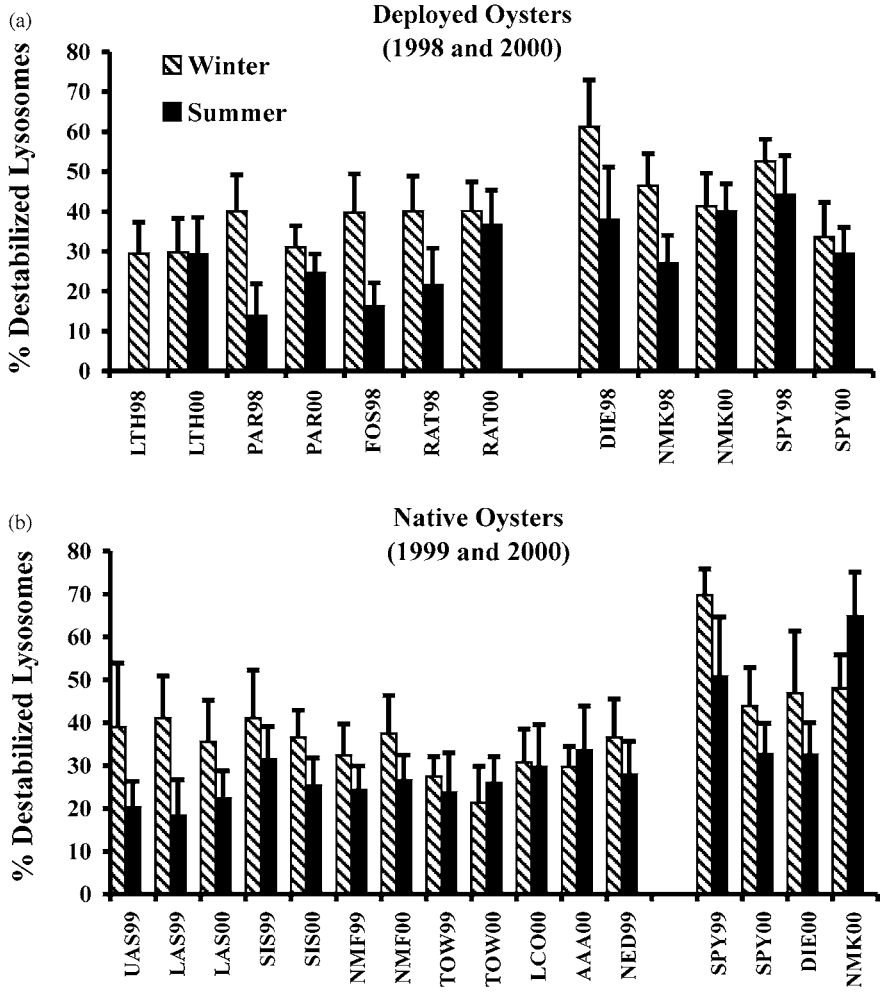

Fig. 1. Lysosomal destabilization rates in oysters, *Crassostrea virginica*, during winter and summer seasons. Results are means + standard deviation ($n = 15$–20 individual oysters) for (a) Juvenile oysters deployed in cages in situ for 28–30 days, and (b) native oysters. The reference or less contaminated sites are grouped to the left, and the highly contaminated sites are grouped together to the right.

signs of perturbation during both seasons, and were often much higher during the winter. There were many sites that were much less contaminated that had seasonal differences that may reflect subtle but important information about habitat status. Many of these sites had no major inputs except those associated with suburbanization and recreational boating. These kinds of studies provide an important means for identifying the potential effects of contaminant impacts, particularly at areas that are experiencing low level chronic exposures. Seasonal assessments may provide subtle but important information about habitat condition and potential contaminant impacts. An appreciation of the underlying causes associated with seasonal

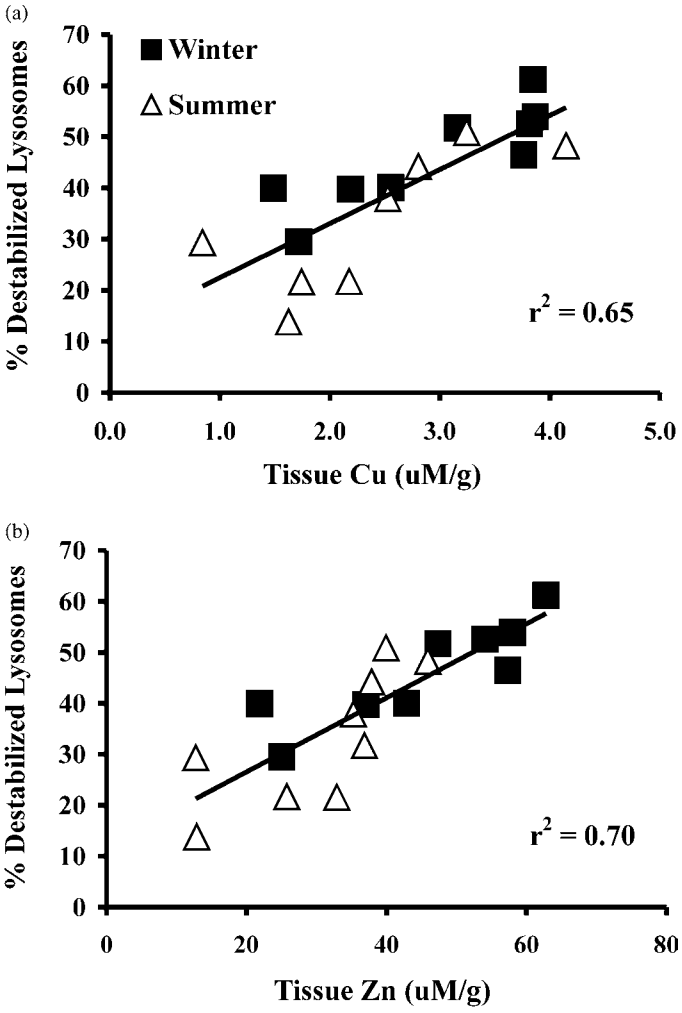

Fig. 2. Plots of the regression analyses of tissue metal concentrations and lysosomal destabilization rates for Cu and Zn of juvenile oysters deployed in cages in situ for 28–30 days during the winter and summer of 1998.

differences may enable us to use lysosomal assays to facilitate the identification of early signs of contaminant stress.

Acknowledgements

This work was funded by US EPA STAR Grant No. R826201 and NOAA CICEET Grant No. NA870R0512, but has not been subjected to review and therefore does not necessarily reflect the views or any official endorsement of these Agencies. This is SCDNR Marine Resources Research Institute Publication #484.

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